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G-protein-coupled receptor genes as protooncogenes: Constitutively activating mutation of the α_{1B}-adrenergic receptor enhances mitogenesis and tumorigenicity

(catecholamines/transformation/atherogenesis)

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ABSTRACT **The α_{1B}-adrenergic receptor (α_{1B}-ADR) is a member of the G-protein-coupled family of transmembrane receptors. When transfected into Rat-1 and NIH 3T3 fibroblasts, this receptor induces focus formation in an agonist-dependent manner. Focus-derived, transformed fibroblasts exhibit high levels of functional α_{1B}-ADR expression, demonstrate a catecholamine-induced enhancement in the rate of cellular proliferation, and are tumorigenic when injected into nude mice. Induction of neoplastic transformation by the α_{1B}-ADR, therefore, identifies this normal cellular gene as a protooncogene. Mutational alteration of this receptor can lead to activation of this protooncogene, resulting in an enhanced ability of agonist to induce focus formation with a decreased latency and quantitative increase in transformed foci. In contrast to cells expressing the wild-type α_{1B}-ADR, focus formation in "oncomutant"-expressing cell lines appears constitutively activated with the generation of foci in unstimulated cells. Further, these cell lines exhibit near-maximal rates of proliferation even in the absence of catecholamine supplementation. They also demonstrate an enhanced ability for tumor generation in nude mice with a decreased period of latency compared with cells expressing the wild-type receptor. Thus, the α_{1B}-ADR gene can, when overexpressed and activated, function as an oncogene inducing neoplastic transformation. Mutational alteration of this receptor gene can result in the activation of this protooncogene, enhancing its oncogenic potential. These findings suggest that analogous spontaneously occurring mutations in this class of receptor proteins could play a key role in the induction or progression of neoplastic transformation and atherosclerosis.**

Cellular oncogenes originate by the activation of normal cellular genes with latent transforming potential (i.e., protooncogenes) and subvert key regulatory pathways controlling cell proliferation. Several oncogenes have been shown to encode for altered receptor proteins, including c-*fms*, *erbB*, *kit*, *neu*, *ros*, and *mas* (1–4), and some have also been associated with human malignancies (5–7). The known cellular homologues of these mutant receptors belong to different receptor classes based on their structural features and coupling to specific signal transduction pathways. The *mas* oncogene, for example, bears structural homology to the broad superfamily of guanine nucleotide-binding protein (G-protein)-coupled receptors (8); transmembrane receptors that transduce extracellular stimuli through G-protein intermediates.

Multiple mechanisms exist for modulating the transforming potential of protooncogenes including mutational change; e.g., a point mutation in the *ras* family of protooncogenes converts them to actively transforming oncogenes (9, 10). Such mutations can activate protooncogenes by altering their level of expression or by modifying key regulatory domains, resulting in constitutive activity.

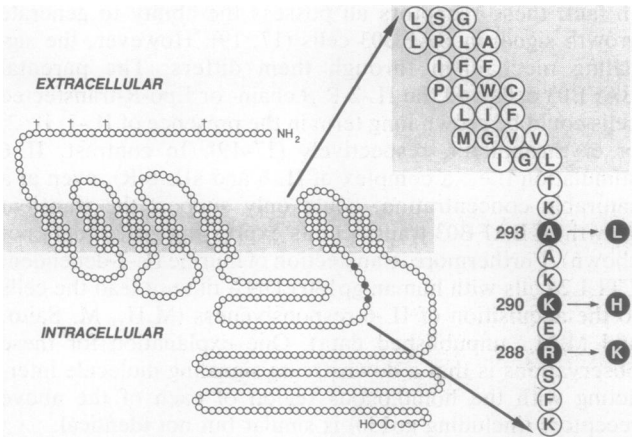

FIG. 1. Structural model of the α_{1B}-ADR, with an enlarged region illustrating the carboxyl terminus of the third intracellular loop and the sixth transmembrane domain. Solid circles indicate the amino acids of the wild-type α_{1B}-ADR that were mutated (positions 288, 290, and 293); the amino acid residues replaced in the α_{1B}-ADR mutant are shown to the right. Crosses represent potential glycosylation sites near the amino terminus.

We previously reported (11) that mutational alteration of one of the adrenergic receptors (Fig. 1), the α_{1B}-adrenergic receptor (α_{1B}-ADR), markedly alters agonist binding affinity and potency in activating intracellular signaling pathways (11). This receptor is a member of the G-protein-coupled receptor superfamily (12) and activates phosphatidylinositol (PI) hydrolysis; a signaling pathway that appears to play a crucial role in mitogenesis (13, 14). Substitution of residues at the carboxyl terminus of the third intracellular loop of the α_{1B}-ADR ($Arg^{288} \rightarrow Lys$, $Lys^{290} \rightarrow His$, and $Ala^{293} \rightarrow Leu$) results in an increase in both the binding affinity of norepinephrine (NE) and its potency to stimulate PI hydrolysis by 2–3 orders of magnitude. In addition, this activating mutation appears to render the α_{1B}-ADR constitutively active, resulting in the stimulation of PI hydrolysis in the absence of agonist-induced receptor activation. This effect was observed in cells expressing the α_{1B}-ADR mutant in both the presence and the absence of serum (11), excluding receptor activation by small quantities of endogenous catecholamines. Furthermore, single amino acid substitutions at residue 293

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Abbreviations: ADR, adrenergic receptor; PI, phosphatidylinositol; [^{125}I]HEAT, 2-{β-(4-hydroxy-3-[^{125}I]iodophenyl)ethylaminomethyl}-tetralone; NE, norepinephrine.

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appear sufficient to increase the affinity and potency of NE and to induce constitutive activity (11, 30). Several subfamilies of G-protein-coupled receptors that also activate PI hydrolysis, the serotonin (1C) and muscarinic cholinergic (m1, m3, and m5) receptors, have been shown to result in conditional, agonist-dependent transformation (15, 16). Therefore, we investigated whether constitutively activating mutations, such as the one we described for the α_{1B}-ADR, might serve as a mechanism for activating G-protein-coupled receptor protooncogenes, thereby enhancing their ability to subvert normal signaling pathways, and result in neoplastic transformation independent of agonist supplementation.

MATERIALS AND METHODS

Cell Culture. Rat-1 fibroblasts were grown in Dulbecco's modified Eagle's medium (DMEM) containing 5% (vol/vol) fetal bovine serum and gentamicin (100 μg/ml). For selection, cells were cultured in the presence of a neomycin analogue, G418 sulfate (300 μg/ml; GIBCO).

Expression Plasmids. The cDNAs of the hamster α_{1B}-ADR (17), α_{1B}-ADR mutant (11), and activated Ha-*ras* were subcloned by blunt-ended ligation into the *Bam*HI site of the retroviral expression vector pZIP-NeoSV(X)1 (18). This recombinant plasmid contains strong promoter and enhancer elements in the Moloney murine leukemia virus long terminal repeats and carries the selectable drug-resistance gene for aminoglycoside phosphotransferase.

DNA Transfection. Subconfluent cells were transfected with 10 μg of the recombinant expression plasmids by the calcium phosphate precipitation method (19). After 16 hr, the DNA precipitate was removed, and the cells were washed and then grown for 24 hr in complete medium.

Focus-Formation Assays. Rat-1 fibroblasts were seeded at a density of 2×10^5 cells in 100-mm dishes. Cells were grown overnight, then transfected with expression vector alone or containing the α_{1B}-ADR, α_{1B}-ADR mutant, or activated Ha-*ras* cDNA inserts as described above. The following day, they were split 1:5, allowed to attach overnight, and then cultured in the presence or absence of 10 μM NE in DMEM with 5% fetal bovine serum. Dishes were supplemented with NE daily, fed every 3 days with fresh medium, and scored after 4 weeks of culture.

Ligand Binding. Ligand binding was assayed on membranes prepared from stably transfected Rat-1 fibroblast cell lines (20). For saturation binding analysis, concentrations of 2-{β-(4-hydroxy-3-[^{125}I]iodophenyl)ethylaminomethyl}tetralone ([^{125}I]HEAT) ranged from 10 to 500 pM, and nonspecific binding was determined using 1 μM prazosin; in competition experiments, the radioligand concentration was 100 pM. Ligand binding parameters were determined using computerized iterative nonlinear regression analysis (21). For ligand binding studies on tumor tissue, animals were sacrificed and tissue was rapidly excised and frozen in liquid nitrogen. Tissue fragments were resuspended in 50 mM Tris buffer (pH 7.4) containing 150 mM NaCl, 5 mM EDTA, trypsin inhibitor (10 μg/ml), leupeptin (10 μg/ml), and bacitracin (200 μg/ml) and were homogenized with a Polytron (Brinkmann) at maximum speed. The particulate fraction was pelleted by centrifugation at 19,000 × *g* for 10 min, washed once, and Dounce homogenized, and the membranes were used for ligand binding.

Inositol Phosphate Determination. Cells were plated in 30-mm dishes and incubated overnight with *myo*-[2(N)-^{3}H]inositol (3 μCi/ml; 1 Ci = 37 GBq; New England Nuclear) in DMEM containing 3.3% fetal bovine serum, as described (11). After stimulation, inositol phosphates were extracted, and separated on AG 1-X8 anion-exchange columns (Bio-Rad). Total inositol phosphates were eluted with 1 M ammonium formate/0.1 M formic acid.

Cell Growth. Cells were plated in 100-mm dishes at a density of 5×10^5 cells per dish in DMEM containing 5% fetal bovine serum. After 24 hr, half the dishes received daily supplementation with 10 μM NE, and all the dishes received fresh medium every 2–3 days. At each time point, cells were trypsinized and cell counts were determined with an electronic particle counter (Coulter).

Tumorigenesis. Rat-1 fibroblasts and G418-selected fibroblast cell lines were cultured in the presence of 10 μM NE for 14 days. Cells were trypsinized, washed, and resuspended in serum-free DMEM, and then 5×10^6 cells were injected at two sites in 21- to 28-day-old female *nu/nu* CD-1 mice (Charles River Breeding Laboratories). Animals were monitored at 2- to 3-day intervals for the development of tumors over a 4- to 8-week period.

RESULTS AND DISCUSSION

α_{1B}-ADR Expression Results in Agonist-Dependent Focus Formation. The functional consequences of receptor expression on cell growth were assessed after transfection of Rat-1 fibroblasts with the α_{1B}-ADR recombinant plasmid. In the absence of added agonist, no change in phenotype or focal cell overgrowth was observed after 4 weeks in culture (Fig. 2*A*). The cells formed a uniform monolayer in culture and became arrested through contact inhibition. However, agonist (NE) stimulation reproducibly resulted in the induction of the transformed phenotype with focus formation in confluent monolayers within 2–3 weeks (Fig. 2*B*). Within foci, cells manifested the malignant phenotype with the loss of normal density-dependent growth inhibition, resulting in increased cellular packing, refractility, and a disordered cellular orientation. Focus formation was strictly agonist-dependent and was not observed in untransfected fibroblasts or those transfected with expression vector alone. The initial frequency of agonist-induced focus formation in Rat-1 fibroblasts was low when compared with focus formation resulting from transfection with the activated *ras* oncogene (Table 1). Agonist-induced foci represented 15–25% of transfected clones with the number of G418-resistant colonies (40–60 per dish) assessed in duplicate transfections. Parallel experiments employing NIH 3T3 mouse fibroblasts resulted in a marked increase in the frequency of agonist-induced focus formation (Table 1) with no significant change in the transfection efficiency. These cells, however, also exhibited a high background of spontaneous transformation in control and untreated cells.

The ability of catecholamines to induce neoplastic transformation of fibroblasts transfected with the α_{1B}-ADR suggests a role for the receptor in regulating mitogenesis and identifies its gene as a protooncogene. While oncogenes, the activated form of protooncogenes, are capable of inducing transformation with high efficiency, their precursors are less

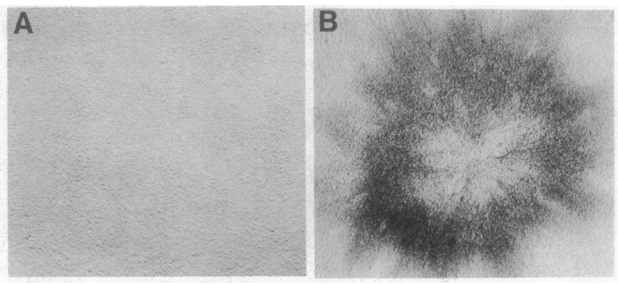

FIG. 2. Agonist-dependent transformation of Rat-1 fibroblasts transfected with α_{1B}-ADR cDNA. Rat-1 fibroblasts were transfected with a retroviral expression vector, pZIP-NeoSV(X)1, containing the α_{1B}-ADR cDNA. Cells were then cultured for 4 weeks in the absence (*A*) or presence (*B*) of 10 μM NE. (×≈6.)

Table 1. Focus formation in Rat-1 and NIH 3T3 fibroblasts transfected with expression vector pZIP-NeoSV(X)1 alone or containing the α_{1B}-ADR, α_{1B}-ADR mutant, or *ras* cDNA inserts

	No. of foci			
	Rat-1		NIH 3T3	
DNA	−NE	+NE	−NE	+NE
Vector	0	0	10	12
α_{1B}	0	8	12	85
α_{1B} mutant	0	11	ND	ND
ras	206	197	160	180

Cells were cultured in the presence or absence of 10 μM NE, as described in *Materials and Methods*. The number of transformed foci was scored after 4 weeks of culture. Results represent the average of duplicate determinations from two independent experiments, which agreed within 25%. ND, not determined.

competent or may even be incompetent. Thus, the number of foci initially generated by constant agonist stimulation may be a reflection of the relatively low intrinsic activity of the unactivated receptor protooncogene. Alternatively, *in vitro* transformation may require collaboration of oncogenes (22). Agonist-induced receptor activation may, therefore, function to complement preexisting activated protooncogenes in these cells, representing a "second hit" phenomenon.

Maintenance of the Transformed Phenotype Requires Continuous Receptor Activation. The dependency of focus formation on both transfection with α_{1B}-ADR cDNA and continuous activation of this receptor by catecholamines implicates the α_{1B}-ADR in activating transmembrane signaling pathways that result in cellular transformation. Multiple cell lines established from individual foci of transformed Rat-1 fibroblasts, however, showed no spontaneous focus formation when maintained in culture in the absence of added agonist (Table 2). On reexposure to agonist, however, these cells showed a markedly enhanced ability for focus generation, inducing transformed foci at significantly increased rates within 2–3 weeks (Table 2). Focus formation remained strictly agonist-dependent and could be inhibited by concurrent administration of the selective α_1-antagonist prazosin (data not shown). The absence of focus formation in unstimulated focus-derived cells provides evidence for the essential role of receptor activation in maintenance of the transformed phenotype.

Morphologically, cells expressing the α_{1B}-ADR exhibited the normal phenotype, when cultured in the absence of catecholamines (Fig. 3*A*). In contrast, the same cells, when grown in the presence of NE, demonstrated an agonist-dependent alteration in cellular morphology with a loss in cellular orientation and decreased adhesiveness (Fig. 3*B*).

Table 2. Focus formation in focus-derived Rat-1 fibroblast lines

	No. of foci	
DNA	−NE	+NE
Vector	0	0
	0	0
α_{1B}	0	75
	0	80
α_{1B} mutant	45	>500, sheets
	65	>500, sheets
ras	>500	>500
	450	460

Cell lines were established from cells transfected with the expression vector pZIP-NeoSV(X)1 alone or from separate transformed foci of Rat-1 fibroblasts transfected with vector containing the α_{1B}-ADR, α_{1B}-ADR mutant, or *ras* cDNAs; two clones are shown for each. Cells were cultured in the presence or absence of 10 μM NE, and the number of foci was scored after 3 weeks. Results represent the mean of duplicate determinations from two independent experiments, which agreed within 20%.

This disordered pattern of growth was blocked by concurrent incubation of the cells with prazosin, which reverted the cells to the untransformed phenotype (Fig. 3*C*). These results confirm the conditional nature of transformation of Rat-1 fibroblasts by the α_{1B}-ADR, a process requiring continuous agonist-induced receptor activation.

Mutational Alteration of the α_{1B}-ADR Results in Protooncogene Activation. Having established that the α_{1B}-ADR could function as a protooncogene, we investigated whether mutational alteration of this receptor (Fig. 1), which induces constitutive activity, would result in protooncogene activation and enhance the oncogenic potential of the α_{1B}-ADR. Transfection of Rat-1 fibroblasts with the expression plasmid containing the α_{1B}-ADR mutant cDNA resulted in malignant transformation with focus formation *in vitro* at initial rates comparable to those of cells expressing the wild-type receptor (Table 1). Restimulation of focus-derived cell lines with agonist, however, resulted in an enhanced ability for focus generation with a quantitative increase in focus number (Table 2); the α_{1B}-ADR mutant-transfected cells attained rates of focus formation comparable to that of the activated *ras* oncogene. In addition, the latency period to focus formation was decreased in these cell lines, with foci appearing within 7 days compared with 14–21 days in cells transfected with the wild-type receptor. Further, while focus formation was markedly augmented by agonist administration, it was not absolutely agonist-dependent with the generation of foci in unstimulated cells (Table 2). Morphologically, these cells formed broad sheet-like plaques of transformed cells, rather than the discrete focal cell overgrowths seen in cells express-

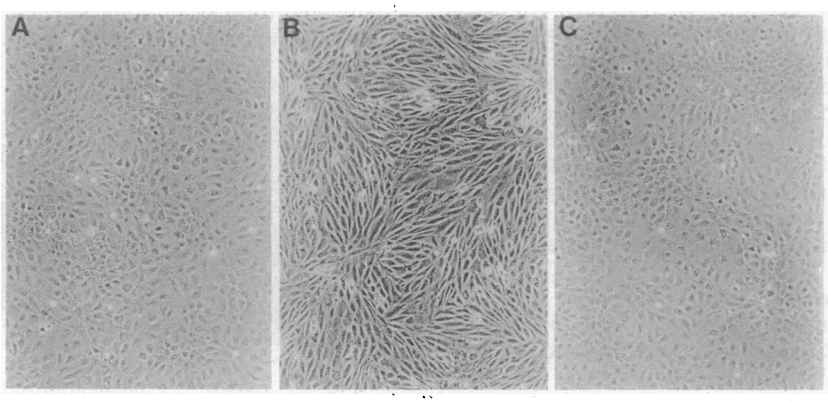

FIG. 3. Agonist-dependent transformation of Rat-1 fibroblasts transfected with the α_{1B}-ADR cDNA. The morphology of a representative Rat-1 fibroblast line derived from a single transformed focus expressing the α_{1B}-ADR is shown. Cells were grown in the absence of added ligand (*A*), in the presence of 10 μM NE (*B*), or in the presence of 10 μM NE and 1 μM prazosin (*C*) for 36 hr. (×≈45.)

Table 3. Parameters of ligand binding and activation of PI hydrolysis in Rat-1 fibroblast lines expressing the α_{1B}-ADR or α_{1B}-ADR mutant

Receptor	Ligand binding* NE K_i, nM	[^{125}I]HEAT K_d, pM	[^{125}I]HEAT B_{max}, pmol/mg	NE-stimulated PI hydrolysis, % increase†
Vector	—	—	0	0
	—	—	0	0
α_{1B}	5815	44.1	5.1	758
	5333	38.3	2.7	410
α_{1B} mutant	42	37.9	2.2	861
	47	46.3	2.6	954

*NE and [^{125}I]HEAT binding were assessed as described in *Materials and Methods*.

†Percent increase of total labeled inositol phosphates over basal levels following stimulation with 10 μM NE for 30 min. Results represent the mean of triplicate determinations of two to three independent experiments, which agreed within 20%.

ing the wild-type α_{1B}-ADR (data not shown). These cell lines, therefore, appear constitutively activated, exhibiting the malignant phenotype independent of agonist supplementation. In addition, NE-induced focus formation is significantly augmented in these lines, reaching the levels observed with the known oncogene *ras*. Because of this enhanced transformed phenotype of the α_{1B}-ADR mutant-transfected cell lines, this mutant receptor was termed the "oncomutant." Conservative mutational alteration of the α_{1B}-ADR, thus, appears to activate the transforming activity of this protooncogene, enhancing its oncogenic potential.

α_{1B}-ADR Overexpression and Activation of Transmembrane Signaling Pathways in Transformed Foci. Cell lines established from foci of transformed Rat-1 fibroblasts exhibited high levels of α_{1B}-ADR expression as assessed by the binding of the α_1-ADR antagonist [^{125}I]HEAT. Receptor concentrations were in the range of 3–5 pmol/mg of protein in various clones (Table 3), representing an ≈10-fold higher level of receptor expression than in tissues normally expressing this receptor. The K_d of [^{125}I]HEAT and K_i of NE were in agreement with values established for this receptor (17). Cell lines derived from foci arising after transfection with the α_{1B}-ADR mutant demonstrated receptor expression at ≈2 pmol/mg of protein (Table 3) and exhibited the enhanced agonist affinity previously reported (11). Untransfected Rat-1 fibroblasts or cells transfected with vector alone, on the other hand, showed no specific binding. Therefore, the cells giving rise to transformed foci overexpressed the α_{1B}-ADR, establishing a role for this receptor in mediating the process of neoplastic transformation.

Since Rat-1 fibroblasts do not normally express the α_{1B}-ADR, their ability to mediate functional coupling of this receptor to PI hydrolysis was investigated. In focus-derived fibroblasts, as in cells that normally express the α_{1B}-ADR, receptor activation resulted in phospholipase C-mediated PI hydrolysis with a 400–800% increase in total inositol phosphates following NE stimulation (Table 3). In cell lines expressing the α_{1B}-ADR mutant, NE induced a 900–1000% increase in inositol phosphates (Table 3); this level of PI hydrolysis was seen only in cells expressing the wild-type receptor with a 2-fold higher level of receptor expression. In contrast, no coupling was observed in cells transfected with vector alone or in wild-type Rat-1 fibroblasts, which lack the receptor. The α_{1B}-ADR, appearing on the surface of Rat-1 fibroblasts following transfection, therefore, functionally couples to PI hydrolysis in these cells. Whether receptor coupling to phospholipase C actually mediates the α_{1B}-ADR's ability to induce malignant transformation in these cells remains speculative, however. Coupling of this receptor to alternative G proteins or effector systems may be responsible for triggering uncontrolled cell proliferation.

Oncomutant-Expressing Cell Lines Exhibit an Enhanced Rate of Mitogenesis. Focus formation represents a morphologic manifestation of agonist-induced transformation and implies the loss of contact inhibition, a normal growth regulatory process. To quantitate receptor-mediated effects on mitogenesis, basal and NE-stimulated growth rates were assessed on cell lines expressing the wild-type or mutant α_{1B}-ADRs. Control, vector-transfected cells showed no significant difference in the rate of cell proliferation in the presence or absence of catecholamines (Fig. 4*A*). In the absence of NE, α_{1B}-ADR-expressing cells grew at nearly control levels, reaching the same saturation density as control cells by day 25. The addition of catecholamines to these cells, however, resulted in an augmented rate of cell proliferation, surpassing control levels by day 12 and reaching a final cell density (day 25) that was 1.9 times control levels (Fig. 4*B*). Thus, catecholamines appear to be competent mitogens in these cells acting through the α_{1B}-ADR. The ability of NE alone to induce an 11-fold increase in DNA synthetic activity in serum-free medium provides additional evidence for a direct mitogenic effect of catecholamines; an effect that could be blocked by prazosin, but not by α_2 (idazoxan or yohimbine) or β (propranolol) adrenergic receptor antagonists (data not shown).

Oncomutant-expressing cell lines demonstrated a more pronounced mitogenic response than was observed in agonist-stimulated cells expressing the wild-type α_{1B}-ADR (Fig. 4*C*). They exhibited a significantly shortened doubling time, with cell counts surpassing control levels earlier (day 8), and a 3-fold increase in final cell density compared with control cells. In addition, proliferative activity in these cell lines was maximally activated even in the absence of catecholamine supplementation. Protooncogene activation, therefore, not only resulted in the loss of contact inhibition but also enhanced the rate of mitogenesis in the focus-derived cell lines.

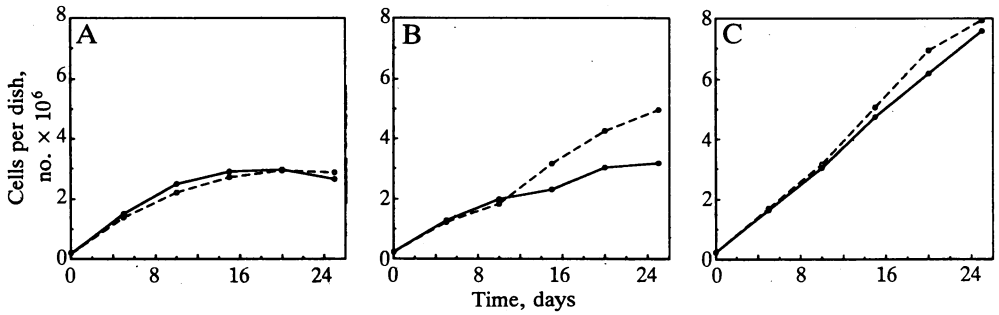

FIG. 4. Growth curves of representative G418-selected Rat-1 fibroblast lines transfected with vector alone (*A*) or expressing the α_{1B}-ADR (*B*) or α_{1B}-ADR mutant (*C*) in the absence (—) or presence (---) of 10 μM NE. Values represent the mean of duplicate determinations of a representative experiment, which agreed within 6%.

The faster doubling time and greater saturation densities of cell lines expressing the α_{1B}-ADR mutant suggests that these cells exhibit a more aggressive malignant phenotype. Mutational alteration of the α_{1B}-ADR, through amino acid substitution in the third cytoplasmic loop, thus appears to result in the activation of this G-protein-coupled receptor protooncogene. Oncomutant-expressing cell lines appear constitutively active with focus formation and maximal growth rates independent of agonist administration.

Transformed Foci Possess Tumorigenic Potential in Nude Mice. *In vitro* assays of transformation do not always translate into tumorigenic potential *in vivo*. *In vivo* tumorigenesis assays employing nude mice provide a more sensitive assay of true oncogenic potential (23). Subcutaneous inoculation of four focus-derived cell lines expressing the α_{1B}-ADR into CD-1 nude mice reproducibly resulted in tumorigenesis with the generation of fibrosarcomas in >90% (11/12) of tested animals after 3 weeks. Four cell lines expressing the α_{1B}-ADR mutant also induced tumors when injected (16/16) and further demonstrated an enhanced ability for tumorigenesis with a decreased period of latency (2 weeks versus 3 weeks), tumor formation in the absence of NE priming, and increased tumor size compared with lines expressing the wild-type receptor. No tumors were observed with injection of Rat-1 fibroblasts or fibroblasts transfected with vector alone after 8 weeks. Examination of the tumor tissue revealed α_{1B}-ADR expression with receptor concentrations in the range of 0.5–6 pmol/mg of protein. Activation of the α_{1B}-ADR, therefore, is able to induce a cascade of biochemical and physiological events that result in the transformation of Rat-1 fibroblasts to cells that are tumorigenic *in vivo*. Mutational activation of this protooncogene augments its oncogenic potential, resulting in a marked enhancement of cell growth and tumorigenesis.

The α_{1B}-ADR Gene Can Function as a Protooncogene and Be Activated by Mutational Alteration. These studies directly demonstrate the potential of the α_{1B}-ADR to activate signal transduction pathways that can abrogate normal growth control mechanisms. Expression of functional α_{1B}-ADRs induces neoplastic transformation of rat and mouse fibroblasts in an agonist-dependent manner to cells possessing tumorigenic potential. Overexpression and agonist-induced activation of the α_{1B}-ADR leading to cellular transformation identifies it as the product of a protooncogene, a cellular gene with a latent potential for inducing neoplastic transformation. The broad distribution and prominent functional role of the α_{1B}-ADR in several body systems [e.g., hepatic regeneration (24)] suggests a potential role for this G-protein-coupled receptor protooncogene in tumorigenesis. In addition, the mitogenic effect of catecholamines on vascular smooth muscle (25, 26) and the ability of the α_{1B}-ADR to modulate cell proliferation may implicate the receptor in the pathogenesis of atherosclerosis.

Mutational alteration of the α_{1B}-ADR that renders it constitutively active appears to activate the transforming potential of this protooncogene and plays an important role in triggering uncontrolled cell proliferation *in vitro*. G-protein-coupled receptors have now emerged as a class of growth factor receptors that can modulate signaling pathways that control cell proliferation (27–29). Given the highly conserved structural features of these receptors and the demonstrated ability of several of them to mediate mitogenesis and transformation (15, 16, 28, 29), the potential exists for a common mechanism of activation that converts these genes to oncogenes. Mutational alteration of key amino acids in the carboxyl terminus of the third intracellular loop, important for receptor/G-protein coupling, may prove to be a generalized mechanism for inducing constitutive activity and activating this class of G-protein-coupled receptor protooncogenes. Spontaneously occurring mutations of this type in this or other structurally related receptor genes *in vivo*, therefore, may subvert the normal function of these receptors and result in human disease states associated with uncontrolled cell growth, including neoplasia and atherosclerosis. In addition, identification of such mutant receptors may provide specific disease markers and pharmacologic targets for therapeutic intervention.

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